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Simple analysis of local anaesthetics in human blood using headspace solid-phase microextraction and gas chromatography–mass spectrometry–electron impact ionization selected ion monitoring

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Abstract

A simple method for analysis of five local anaesthetics in blood was developed using headspace solid-phase microextraction (HS-SPME) and gas chromatography–mass spectrometry–electron impact ionization selected ion monitoring (GC–MS–EI-SIM). Deuterated lidocaine (d_{10}-lidocaine) was synthesized and used as a desirable internal standard (I.S.). A vial containing a blood sample, 5 *M* sodium hydroxide and d_{10}-lidocaine (I.S.) was heated at 120°C. The extraction fiber of the SPME system was exposed for 45 min in the headspace of the vial. The compounds adsorbed on the fiber were desorbed by exposing the fiber in the injection port of a GC–MS system. The calibration curves showed linearity in the range of 0.1–20 μg/g for lidocaine and mepivacaine, 0.5–20 μg/g for bupivacaine and 1–20 μg/g for prilocaine in blood. No interfering substances were found, and the time for analysis was 65 min for one sample. In addition, this proposed method was applied to a medico–legal case where the cause of death was suspected to be acute local anaesthetics poisoning. Mepivacaine was detected in the left and right heart blood samples of the victim at concentrations of 18.6 and 15.8 μg/g, respectively. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Lidocaine; Mepivacaine; Bupivacaine; Prilocaine; Dibucaine

1. Introduction

Local anaesthetics are widely used for various local or regional treatments. These drugs cause occasional medical accidents. An accurate, simple and rapid method for analysis of local anaesthetics is required in forensic practices. The detection of local anaesthetics in human body fluids using liquid–liquid and solid-phase extraction methods has been reported [1–3]. These procedures were laborious and required a long time for one sample. Arthur and Pawliszyn [4] have developed a solid-phase microextraction (SPME) method, which has been used mainly for environmental analysis of organic compounds in water and ground water [5,6]. This technique uses a modified syringe housing a fused-silica fiber coated with a gas chromatographic stationary phase. SPME involves the partitioning of analytes between the fiber and sample matrix, followed by thermal desorption of the analytes into an

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analytical instrument, typically a gas chromatograph. SPME integrates sampling, extraction, concentration and sample introduction. When analytes are extracted from aqueous samples using SPME, two different methods are usually used. One is a headspace (HS) method and the other is a directly immersed method.

Recently, Yashiki et al. [7] and Nagasawa et al. [8] have reported the analysis of amphetamines in urine and blood using HS-SPME. Kumazawa et al. [9] have reported the analysis of local anaesthetics in blood using the HS-SPME. In the method of Kumazawa et al., blood had to be deproteinized prior to analysis. In this study, deuterated lidocaine was synthesized and used as a desirable internal standard (I.S.), and we developed a more simple method using the HS-SPME for analysis of local anaesthetics in blood. This method was applied to a medico–legal case.

2. Experimental

2.1. Materials

A drug-free blood sample collected from a healthy adult male was used to make blood samples containing local anaesthetics, and used as a control blood. Blood samples collected from an autopsy case were kept frozen at −20°C until analyzed.

Prilocaine, bupivacaine, mepivacaine and dibucaine were purchased from Sigma (St. Louis, MO, USA). Lidocaine was purchased from Fujisawa (Osaka, Japan). Decadeuterated diethylamine was purchased from Nippon Sanso (Tokyo, Japan). Other reagents and solvents used were purchased at the highest commercial quality, and used without further purification. All aqueous solutions were prepared using deionized water.

Stock standard solutions (0.1 mg/ml) of local anaesthetics and d_{10}-lidocaine were dissolved in methanol and stored at 4°C in a refrigerator. Some of the original solution stood at room temperature for three months and remained stable. Therefore, we concluded that the refrigerated sample also remained stable.

An SPME system with a replaceable extraction fiber, coated with 100 μm polydimethylsiloxane, was purchased from Supelco (Japan).

2.2. Synthesis of deuterated lidocaine

Decadeuterated lidocaine [d_{10}-lidocaine; 2-(diethyl-d_{10}-amino)-*N*-(2,6-dimethylphenyl)acetamide] was synthesized according to the procedure for synthesizing lidocaine [10]. To synthesize d_{10}-lidocaine, decadeuterated diethylamine [$NH(C_2D_5)_2$] was used instead of diethylamine [$NH(C_2H_5)_2$]. The structural formula of d_{10}-lidocaine is shown in Fig. 1.; m.p. 64°C, ^{1}H NMR ($CDCl_3$) δ 2.23 (6H, s, $-CH_3$), 3.21 (2H, s, $-CH_2-$), 7.08–7.10 (3H, m, aromatic hydrogen), isotopic enrichment 99 atom%,

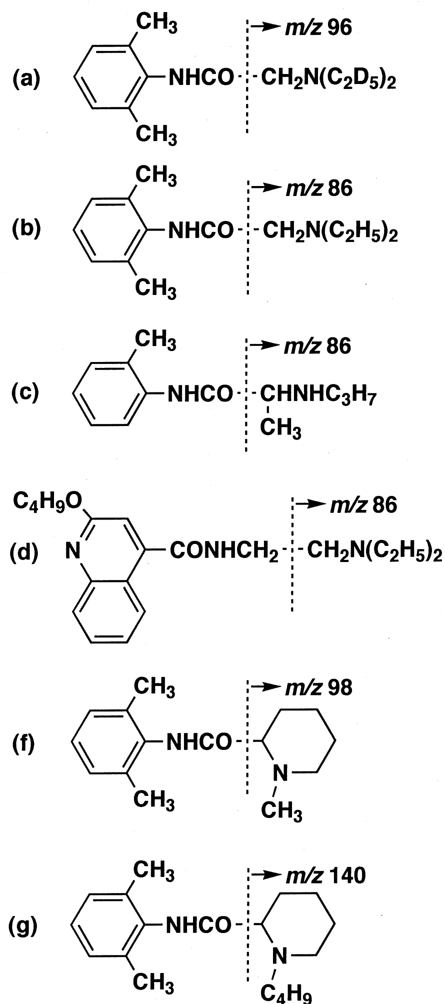


Fig. 1. Structural formulas and hypothetical structures of fragments monitored. (a) d_{10}-Lidocaine, (b) lidocaine, (c) prilocaine, (d) dibucaine, (e) mepivacaine, (f) bupivacaine.

IR (KBr pellet) 3252, 2224, 2053, 1665, 1499, 1424, 1373, 1333, 1285, 1231, 1186, 1163, 1051, 766, 505 cm^{-1}. ^1H (500 MHz) NMR spectrum at 35.0±0.1°C was recorded on a JEOL LA500 spectrometer. Tetramethylsilane was used as an internal reference for ^1H NMR measurement. Isotopic enrichment was calculated for ^1H NMR spectrum. The IR spectrum was recorded on a Shimadzu FTIR-4200 spectrophotometer at 25±2°C.

2.3. Gas chromatography–mass spectrometry

The gas chromatography–mass spectrometry (GC–MS) system used was a Shimadzu QP-5000, equipped with a 30 m×0.32 mm I.D. fused-silica capillary column (J&W, DB-1, film thickness 0.25 μm). The column temperature was set at 100°C for 5 min, and then it was programmed to heat from 100°C to 280°C at 20°C/min. The temperatures of the injection port and the interface were set at 250 and 230°C, respectively. Splitless injection mode was used. Helium with a flow-rate of 1.8 ml/min was used as a carrier gas. Ions used for selected ion monitoring (SIM) and quantitation were m/z 86 for lidocaine, prilocaine and dibucaine, m/z 98 for mepivacaine, m/z 140 for bupivacaine and m/z 96 for d_{10}-lidocaine (I.S.). Hypothetical structures of fragments monitored are shown in Fig. 1.

2.4. Preparation for analysis of local anaesthetics by HS-SPME

A blood sample (0.2 g), d_{10}-lidocaine (0.1 mg/ml, 5 μl) as an internal standard, and sodium hydroxide (5 *M*, 0.8 ml) were placed into a 12-ml vial, and sealed rapidly with a silicone septum and an aluminum cap. Immediately after the vial was heated at 120°C in an aluminum block heater (Dry Thermo Unit TAH-1, Taitec), the needle of the SPME device containing an extraction fiber was inserted through the septum of the vial and the extraction fiber was exposed in the headspace of the vial. After 45 min, the needle was removed from the vial and inserted into the injection port of the GC–MS system. The compounds adsorbed on the fiber were desorbed and analyzed by exposing the fiber for 5 min in the injection port.

2.5. Heating temperature, preheating time and exposure time

To determine the effect of heating temperature, preheating time and exposure time, a blood sample containing 1 μg/g of lidocaine, prilocaine, bupivacaine and mepivacaine and 5 μg/g of dibucaine was prepared and analyzed. The SPME condition used was a modified procedure for local anaesthetics [9]. In the original procedure, the vial was heated at 100°C for 15 min. The fiber was then exposed for 40 min and analyzed.

2.6. Recovery of local anaesthetics under various conditions

To determine extraction recoveries, blood samples spiked with five local anaesthetics at the concentration of 1 μg/g were prepared and analyzed using the above procedure. Extraction recoveries were calculated by comparison with the direct injection of these compounds into the chromatographic system.

2.7. Calibration curve

To determine calibration curves, blood samples spiked with five local anaesthetics at concentrations ranging from 0.01 to 20 μg/g were prepared and analyzed using the above procedure. The calibration curves were obtained by plotting the peak area ratio between local anaesthetics and d_{10}-lidocaine (I.S.).

2.8. Reproducibility

Reproducibility was evaluated by analyzing blood samples containing two different concentrations (1 and 10 μg/g) of five local anaesthetics on the same day (intra-day reproducibility) and over five consecutive days (inter-day reproducibility).

3. Results and discussion

3.1. Heating temperature in the HS-SPME method

The sample vials were preheated for 10 min and the fiber was exposed for 30 min at four different temperatures (80, 100, 120 and 140°C). The ad-

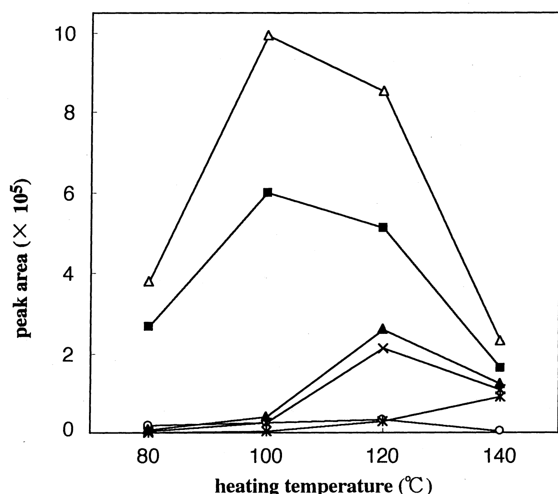


Fig. 2. Plots of the adsorbed amount of local anaesthetics and d_{10}-lidocaine with the heating temperature of the sample vial. (■) Lidocaine, (▲) mepivacaine, (×) bupivacaine, (○) prilocaine, (*) dibucaine, (△) d_{10}-lidocaine.

sorbed amounts of five local anaesthetics and d_{10}-lidocaine are shown in Fig. 2. The adsorbed amounts of lidocaine and d_{10}-lidocaine were maximum at 100°C and those of prilocaine, mepivacaine and bupivacaine were maximum at 120°C. But the adsorbed amount of dibucaine increased as the temperature increased. Although the adsorbed amounts of lidocaine and d_{10}-lidocaine were maximum at 100°C, substantial amounts (ca. 85% of maximum amount) were extracted into the fiber at 120°C, and the adsorbed amounts of mepivacaine and bupivacaine at 100°C were substantially less than those at 120°C. Therefore, the temperature of 120°C was adopted.

3.2. Preheating time in the HS-SPME method

When volatile chemicals are extracted by HS-SPME methods, sample vials are generally put on a heating block for over 20 min before the HS-SPME extraction [11–15]. But no attention has been given to the requirement of this preheating step.

To determine the effect of preheating time on the adsorbed amount of five local anaesthetics and d_{10}-lidocaine, vials were heated at 120°C for four different times (0, 10, 20 and 30 min) before the HS-SPME extraction. And then the fiber was exposed for 30 min. No correlation of the adsorbed amount of those compounds with the preheating time was observed.

3.3. Exposure time in the HS-SPME method

To determine the effect of exposure time on the adsorbed amount of five local anaesthetics and d_{10}-lidocaine, the fiber was exposed for six different times (10, 20, 30, 45, 60 and 90 min). Immediately after the vial was heated at 120°C, the fiber was passed through the septum. The results are shown in Fig. 3. The adsorbed amount of lidocaine, prilocaine and d_{10}-lidocaine reached a maximum within 60 min and decreased over 60 min. For a longer exposure time, the fiber was heated and analytes were desorbed from the fiber to the headspace. Thus, the amount on the fiber decreased. The adsorbed amounts of mepivacaine and bupivacaine were maximum at 60 and 90 min, respectively. The adsorbed amount of dibucaine increased as the exposure time increased. The suitable exposure time of 45 min was used, because the exposure time of 45 min gave

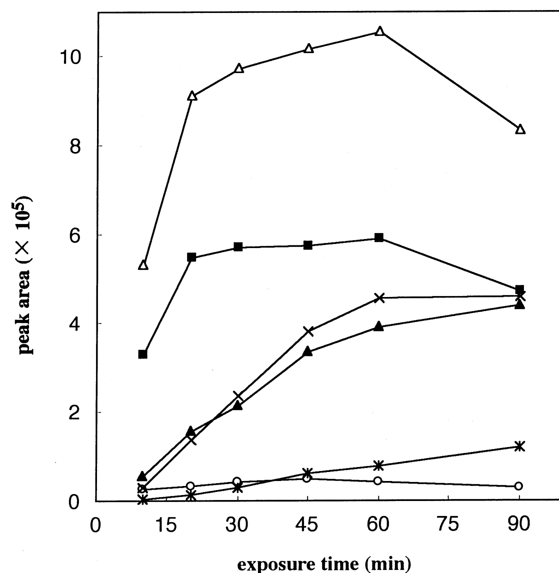


Fig. 3. Plots of the adsorbed amount of local anaesthetics and d_{10}-lidocaine with the exposure time of the extraction fiber of the SPME device in the gas phase. (■) Lidocaine, (▲) mepivacaine, (×) bupivacaine, (○) prilocaine, (*) dibucaine, (△) d_{10}-lidocaine.

Table 1
Recovery of local anaesthetics in the presence of 1 *M* NaOH, 1 *M* NaOH plus $(NH_4)_2SO_4$ and 5 *M* NaOH ($n=5$)

Drug	Recovery of drugs (mean±S.D., %)		
	Composition of mixture		
	0.2 g Blood +0.8 ml 1 *M* NaOH	+0.8 ml 1 *M* NaOH +1 g $(NH_4)_2SO_4$	+0.8 ml 5 *M* NaOH
Lidocaine	1.86±0.03	0.25±0.01	3.79±0.05
Mepivacaine	0.79±0.02	0.49±0.09	5.28±0.35
Prilocaine	1.10±0.01	0.35±0.03	2.90±0.08
Bupivacaine	5.29±0.36	0.48±0.06	8.46±0.27
Dibucaine	0.38±0.07	ND	0.65±0.10

ND=Not detectable.

Table 2
Summarization of results of the calibration curves for determining local anaesthetics in blood ($n=2$)

Drug	Equation of calibration curve[a]	r^2 value[b]	Linearity (μg/g)	LOQ[c] (μg/g)	LOD[d] (μg/g)
Lidocaine	$y=0.366x+0.0482$	0.9993	0.1–20	0.1	0.05
Mepivacaine	$y=0.337x+0.0297$	0.9990	0.1–20	0.1	0.05
Prilocaine	$y=0.172x-0.161$	0.9991	1.0–20	1.0	0.25
Bupivacaine	$y=0.390x-0.0914$	0.9997	0.5–20	0.5	0.01
Dibucaine	Not linear	–	–	–	0.5

[a] y=Peak area ratio (local anaesthetic/d_{10}-lidocaine); x=drug concentration (μg/g).
[b] Correlation coefficient of calibration curve.
[c] Limit of quantitation.
[d] Limit of detection.

sufficient amount to analyze and had a small coefficient of variation.

3.4. Recovery of local anaesthetics under various conditions

The recoveries of five local anaesthetics are shown in Table 1. The recoveries of five local anaesthetics in the presence of 5 *M* sodium hydroxide were higher than those of the other conditions. Therefore, the analysis was performed using 5 *M* sodium hydroxide. But ester-type local anaesthetics such as procaine, tetracaine, benoxinate and T-cain could not be analyzed using this procedure. This is because these drugs are hydrolyzed by strong alkaline solutions [3].

Table 3
Coefficients of variation of intra-day and inter-day measurements for 1 μg/g and 10 μg/g local anaesthetics in blood ($n=5$)

Drug	Coefficients of variation (%)			
	1 μg/g		10 μg/g	
	Intra-day	Inter-day	Intra-day	Inter-day
Lidocaine	1.3	1.8	0.7	1.4
Mepivacaine	6.5	6.7	5.1	5.6
Prilocaine	2.9	4.3	1.8	2.6
Bupivacaine	3.2	4.5	2.3	8.3
Dibucaine	15	26	16	25

3.5. Calibration curve and coefficients of variation in the HS-SPME method

Detailed data of the calibration curves for five local anaesthetics in blood are shown in Table 2. The calibration curves showed linearity in the range of 0.1–20 μg/g for lidocaine and mepivacaine, 0.5–20 μg/g for bupivacaine and 1–20 μg/g for prilocaine. The calibration curve for dibucaine was not linear. The correlation coefficients of the calibration curves

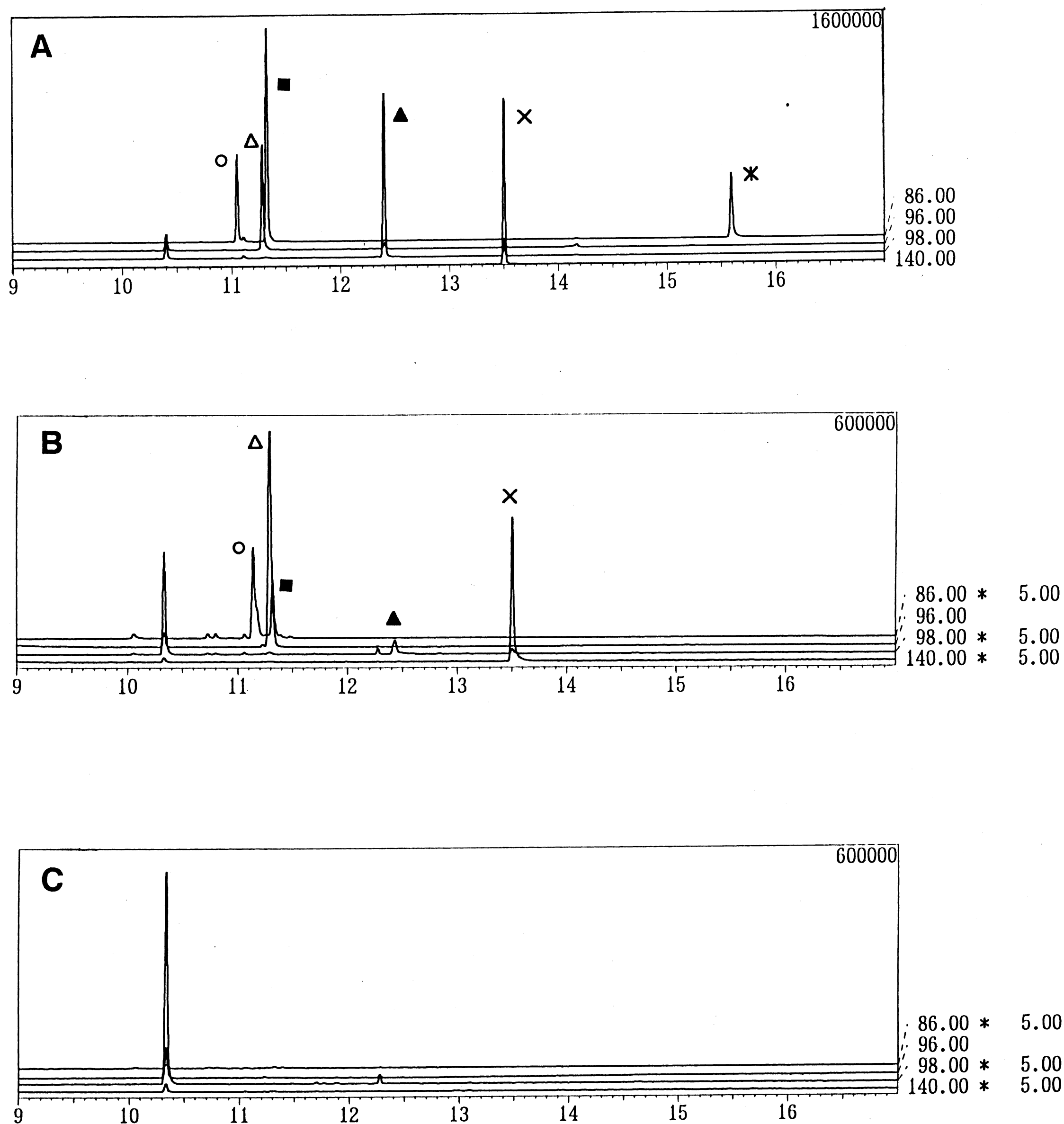
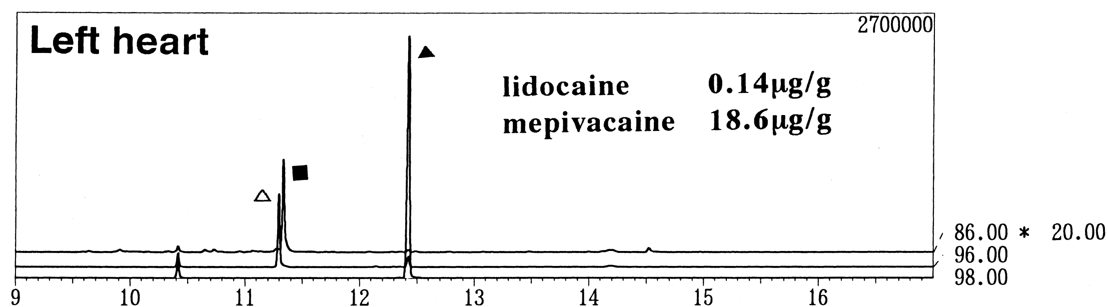
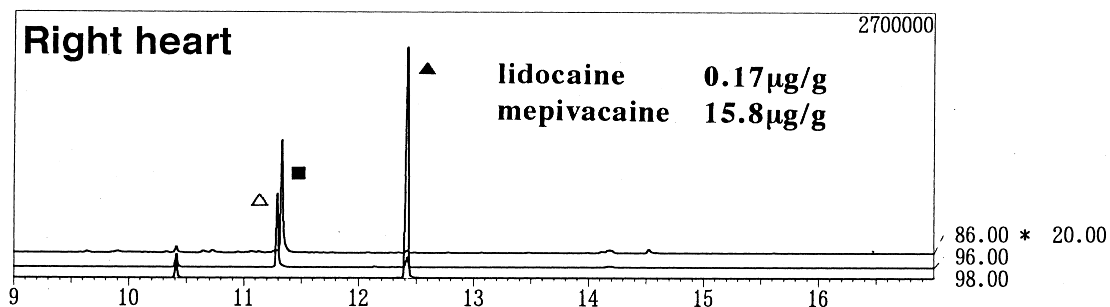
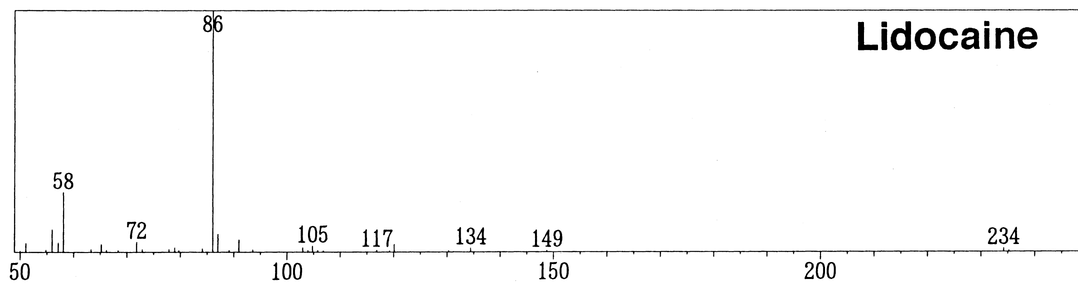


Fig. 4. EI-SIM chromatograms of local anaesthetics in the blood sample. (A) Blood sample containing 5 μg/g lidocaine, mepivacaine, prilocaine and bupivacaine and 10 μg/g dibucaine. (B) LOQ chromatogram: blood containing 0.1 μg/g lidocaine and mepivacaine, 0.5 μg/g bupivacaine and 1 μg/g prilocaine. (C) Blank blood. (○) Prilocaine, (■) lidocaine, (▲) mepivacaine, (×) bupivacaine, (*) dibucaine, (△) d_{10}-lidocaine (I.S.).







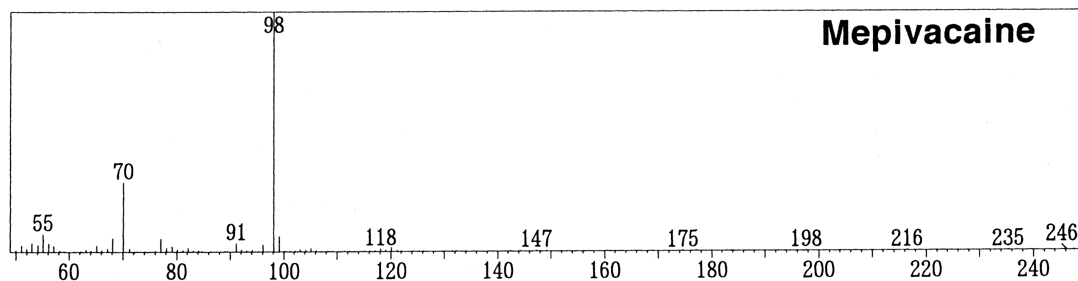


Fig. 5. EI-SIM chromatograms, EI-mass spectra and concentrations of lidocaine and mepivacaine in the blood samples of a medico–legal case. (■) Lidocaine, (▲) mepivacaine, (△) d_{10}-lidocaine (I.S.).

were 0.9990 to 0.9997 for four local anaesthetics. The limit of detection (LOD) was determined at a signal-to-noise ratio of 3 ($S/N=3$). The LODs in blood were 0.01 μg/g for bupivacaine, 0.05 μg/g for lidocaine and mepivacaine, 0.25 μg/g for prilocaine and 0.5 μg/g for dibucaine. The values of the coefficients of variation are shown in Table 3. The intra-day and inter-day coefficients of variation were 0.7–6.5% and 1.4–8.3%, respectively. Not only the toxic levels but the therapeutic levels [16] of four local anaesthetics in blood could be measured using our present method.

3.6. Chromatograms

EI-SIM chromatograms by our present method are shown in Fig. 4. No impurity peak overlapped the peak of these compounds. The retention times were 11.1, 11.3, 12.4, 13.5, 15.6 and 11.3 min for prilocaine, lidocaine, mepivacaine, bupivacaine, dibucaine and d_{10}-lidocaine (I.S.), respectively.

3.7. A medico–legal case

A toxicological analysis of local anaesthetics in human whole blood obtained from a suspected victim of local anaesthetics poisoning was performed by the proposed HS-SPME method. Sharp peaks of local anaesthetics were obtained without disturbance of endogenous interferences. EI-SIM chromatograms, EI-mass spectra and concentrations of local anaesthetics from the left and right heart blood samples of the victim are shown in Fig. 5. Mepivacaine and lidocaine were detected in the left and right heart blood samples of the victim. The concentrations of the left and right heart blood were 18.6 and 15.8 μg/g for mepivacaine, 0.14 and 0.17 μg/g for lidocaine, respectively. The concentration of mepivacaine in blood was higher than the therapeutic level.

4. Conclusion

The HS-SPME method is more simple and rapid than conventional liquid–liquid and solid-phase extractions, and the background of the SIM chromatogram is very clean. The proposed method is very sensitive because of the high adsorbing ability of the SPME system for vaporized drugs. Although sample vials were generally heated to reach thermal equilibrium before the HS-SPME, it was found that this preheating procedure is not necessary when analytes are extracted from blood samples. Compared with the method of Kumazawa et al., a small sample amount (0.2 g) of blood can be analyzed without deproteinization.

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References

[1] H. Seno, O. Suzuki, T. Kumazawa, H. Hattori, Forensic Sci. Int. 50 (1991) 239.
[2] E. Tanaka, Y. Nakagawa, S.X. Zhang, S. Misawa, Jpn. J. Forensic Toxicol. 13 (1995) 11.
[3] M. Terada, M.N. Islam, Z. Tun, K. Honda, C. Wakasugi, T. Shinozuka, J. Yanagida, T. Yamamoto, Y. Kuroiwa, J. Anal. Toxicol. 20 (1996) 318.
[4] C. Arthur, J. Pawliszyn, Anal. Chem. 62 (1990) 2145.
[5] C. Arthur, D. Potter, K. Buchholz, S. Motlagh, J. Pawliszyn, LC·GC 10 (1992) 656.
[6] C. Arthur, L. Killam, S. Motiagh, M. Lim, D. Potter, J. Pawliszyn, Environ. Sci. Technol. 26 (1992) 979.
[7] M. Yashiki, T. Kojima, T. Miyazaki, N. Nagasawa, Y. Iwasaki, K. Hara, Forensic Sci. Int. 76 (1995) 169.
[8] N. Nagasawa, M. Yashiki, Y. Iwasaki, K. Hara, T. Kojima, Forensic Sci. Int. 78 (1996) 959.
[9] T. Kumazawa, X.P. Lee, K. Sato, H. Seno, A. Ishii, O. Suzuki, Jpn. J. Forensic Toxicol. 13 (1995) 182.
[10] N. Loegren, Arkiv Kemi Mineral. Geol. 22A (1946) 18.
[11] J. Tytgat, P. Daenens, Int. J. Legal Med. 109 (1996) 150.
[12] T. Kumazawa, X.P. Lee, M.C. Tsai, H. Seno, A. Ishii, K. Sato, Jpn. J. Forensic Toxicol. 13 (1995) 25.
[13] H. Seno, T. Kumazawa, A. Ishii, M. Nishikawa, K. Watanabe, H. Hattori, O. Suzuki, Jpn. J. Forensic Toxicol. 14 (1996) 30.
[14] S. Li, S.G. Weber, Anal. Chem. 69 (1997) 1217.
[15] M. Nishikawa, H. Seno, A. Ishii, O. Suzuki, T. Kumazawa, K. Watanabe, H. Hattori, J. Chromatogr. Sci. 35 (1997) 275.
[16] R.C. Baselt and R.H. Cravey, Disposition of Toxic Drugs and Chemicals in Man, Chemical Toxicology Institute, CA, 4th ed., 1995.